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SOME FUNDAMENTAL MORPHOLOGICAL OBJECTIONS TO THE MUTATION THEORY OF DE VRIES

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THE hypothesis of the saltatory origin of species has received a new impetus from the investigations of De Vries,* published in his "Mutationstheorie" and subse-

1 To an address delivered in Brussels before the outbreak of the war and published in Science (Vol. 40, No. 1020, July 17th.), Professor de Vries appends a criticism of the writers preliminary article on mutation, likewise published in Science (Vol 39, No. 1005, April 3d.). The gist of his objection to the writer's position, that Œnothera and other members of the Onagraceæ are in a position of hybrid contamination, as evidenced by the frequent sterility or partial sterility of their pollen, is the contention that pollen sterility and gametic sterility in general is not sufficient evidence of hybrid contamination. To this statement two replies may be made. In the first place prominent geneticists for many years have recognized pollen sterility as important evidence of hybridization. Secondly investigations, which have now become very extensive, on the Angiosperms as a whole, show very interesting conditions in many natural families. While the monotypic species and those which are isolated geographically or phenologically (that is by a time of flowering later or earlier than that of the mass of species belonging to the genus) have invariably good pollen, those species, which overlap in their geographical range and in their times of flowering in many cases are characterized by abortion of the reproductive cells. In other words pollen infertility is only found where the possibility of crossing is present. This principle has been illustrated in the body of the present article by reference to the Rosaceæ. Taking a further illustration from the large family Ranunculaceæ, *Ranunculus acris* and *R. repens,* which overlap both in range and time of flowering have pollen, which is often largely imperfect, particularly in the first mentioned species. *R. rhomboideus* on the other hand, flowering in the very early spring has perfect pollen development.

quent works. The chief foundation for his views, in regard to the instantaneous origin of species, is furnished by the conduct of *Œnothera lamarckiana* in cultures. It has been somewhat generally recognized that *O. lamarckiana,* and more recently, other species of the genus as well, constitute crucial evidence in regard to the validity of the mutation hypothesis on the botanical side. A great many investigations on the genetics and cytology of *O. lamarckiana* and other species, as well as crosses between species and "mutants" of *Œnothera,* have been carried on during the past decade by De Vries, and his followers and opponents. As a result a huge and highly technical literature has grown up. *Œnothera* is obviously regarded, on the botanical side at any rate, as the touchstone of the mutation hypothesis as formulated by De Vries. Obviously if this genus does not stand the test of critical investigation, the mutation hypothesis, so far as its validity depends upon De Vries's chosen illustration, is discredited.

Since *Œnothera* and by obvious implication the Onagraceæ, to which it belongs, have become authority for the mutation hypothesis, in its latest revival, they must like Cæsar's wife be beyond suspicion. Like Cæsar, *Œnothera* has become a name of authority and its family affairs accordingly, should be beyond suspicion, when subjected to the most searching investigation. It is apparently just in this direction that the weak spot of the mutation hypothesis lies. Too much attention has apparently been given to ringing the changes on the so-called mutants of *Œnothera* and not enough to the investigation of the general morphological situation in the Onagraceæ, to which this much-discussed genus belongs.

Unusual variability in plants is ordinarily regarded as *prima facie* evidence of hybridism and the suggestion has in fact frequently been made by professional geneticists (*e. g.,* Bateson, Davis, East, Gates and others) that *Œnothera lamarckiana* is a hybrid. It is perhaps of interest in this connection to recall that one of the commonest expedients adopted by the practical breeder, for breaking

up the continuity of the germ plasm, is hybridization. Apposite in this connection is the wholesale hybridizing practised by Burbank, for the purpose of bringing about the necessary genetic plasticity in his cultures and thus obtaining by resultant mutation or variation, new and desirable varieties of useful plants. The morphological peculiarities of hybrids have been clearly recognized for nearly a hundred years. They are for example clearly set down in Gaertner's rare and classic prize essay, entitled "Versuche und Beobacthungen ueber die Bastarderzeugung im Pflanzenreich" (Stuttgart, 1849). Curiously enough these important criteria have been largely ignored by the adherents of the mutation hypothesis of De Vries. A very important and generally observed difference between hybrids and genetically pure species, is the very easily detected one of pollen sterility, partial or complete. Of course when the hybridizing forms show a considerable degree of compatibility, this character may be inconspicuous or even absent. Further even in cases where it is originally present, it may be subsequently largely eliminated by selection. De Vries himself has noted that about one third of the pollen of *O. lamarckiana* is abortive. The English geneticist Bateson was struck with this peculiarity of the species, so much discussed in recent years, in relation to its variable offspring in cultures and promptly and first called attention to the obvious significance of this feature, suggesting that *O. lamarckiana* was a hybrid and that its remarkable conduct was the result of hybridization. This objection has in reality never been met. It is the purpose of the present article to show on grounds commonly accepted by geneticists and morphologists, that not only is genus *Œnothera* in general characterized by genetically impure or hybrid species, but that the condition of genetical impurity is extremely common in the Onagraceæ as a whole.

It will be convenient to begin with the examination of our common and very variable garden *Fuchsias,* which belong to the family Onagraceæ. The common *Fuchsia,*

sometimes known to gardeners as *Fuchsia speciosa,* is recognized as a hybrid derivative of *Fuchsia magellanica,* a native of southern South America. Fig. 1 illustrates

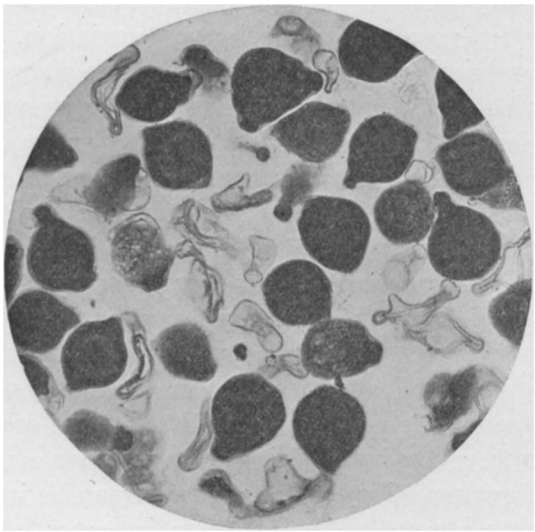

FIG. 1

photomicrographically, the condition of the pollen in one of the garden varieties of *Fuchsia.* The sound pollen grains appear as dark bodies with two or more germination pores projecting from their surfaces. The dark color of the grains is due to the deeply staining character of their protoplasmic contents. More than a third of the pollen present in the anther cavity is abortive and is represented in the photograph by shrivelled light-colored objects, which are in fact empty and collapsed pollen grains. In other varieties of the garden *Fuchsia,* the grains may either be entirely abortive and empty (as is the case for example in the so-called mutant of *Œnothera lamarckiana,* known as *O. lata*) or they may all be more or less well developed so far as their protoplasmic contents are concerned, but extremely varying in size. In the present description, perfection or imperfection of pollen is judged only from the morphological aspect, because this is the significant point of view from the standpoint of the

detection of hybridization. Physiological sterility is frequently due to entirely different causes than genetical lack of harmony, as for example in the horseradish or the potato (*Solanum*). In the former it has been found possible to bring about the formation of fertile seed by simply girdling the top of the subterranean storage region of the plant, so as to prevent the undue descent of assimilates. The common white lily, *Lilium candidum,* presents a similar condition, for here the setting of seed takes place only when the leafy flowering axis is severed from its bulb and kept in water. So far as I am aware, there have been no experiments as to the result of severing the continuity of the phloem (girdling), in relation to the restoration of seed production in the potato. The common yellow day lily (*Hemerocallis*) possibly presents a case similar to that of *Lilium candidum,* for it does not ordinarily set seed, although in all the examples I have examined the pollen was morphologically perfect. I have not yet been able to secure flowers of any pure species of *Fuchsia,* a genus which flourishes mostly in the remoter parts of South America and in the New Zealand islands. The cultivation of *Fuchsias,* although once very popular, has now gone out of vogue and it is consequently difficult to secure specimens of the species. As has been pointed out the commonly cultivated *Fuchsias* are of hybrid origin.

We may now turn our attention to a very puzzling genus of the Onagraceæ, namely *Epilobium.* This genus has been a great riddle to systematists and the determination of species has been extremely difficult on account of their extreme variability. In European systematic works, this high degree of variability is recognized clearly to be largely due to hybridization and in such a standard work as the "Naturliche Pflanzenfamilien" of Engler and Prantl, the statement is definitely made that the various species of *Epilobium* frequently and commonly hybridize with one another in nature. Let us consider in this connection the northern hemisphere cosmopolitan species, known as *Epilobium angustifolium,* the willow herb or

fire weed, which by contrast to many of the other *Epilobiums,* is so constant and distinct that it is frequently referred to a separate genus, *Chamænerion.* This species shows its most marked distinction from other species of *Epilobium* (*Epilobium* proper) in the fact that its pollen grains are separate and not in tetrads, as is the case in other common species. Fig. 2 reproduces photograph-

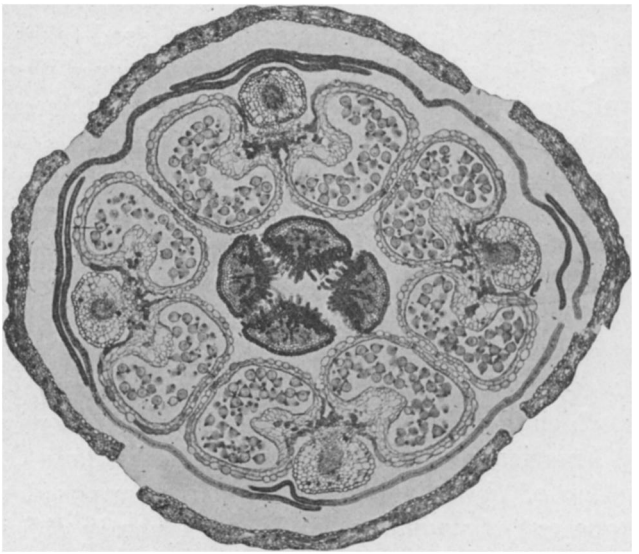

FIG. 2

ically a transverse section of a mature flower bud of *E.* (*Chamænerion*) *angustifolium.* On the outside are seen the floral envelopes, two in number, composed, as is the rule in the Onagraceæ, of four parts each. Within lie four stamens represented by their anther sacks and internal to these are four stigmas representing the carpellary or ovarial portion of the flower. The photograph is on a sufficient scale of magnification to show the pollen grains in the loculaments or cavities of the anthers. Obviously the pollen is very uniform and perfect in its development. Fig. 3, likewise photographic, illustrates the organization of the pollen as viewed with a much higher magnification of the microscope. Although some of the grains are only

partially included in the plane of section, it is quite clear, that like those of *Fuchsia,* figured above, they have projecting germination pores, but unlike the *Fuchsia* of our illustration, all the pollen grains of *Epilobium* (*Chamænerion*) *angustifolium* are perfectly developed. I have examined the pollen of the species under discussion from widely separate geographical regions and under different conditions of growth and season, with the uniform result,

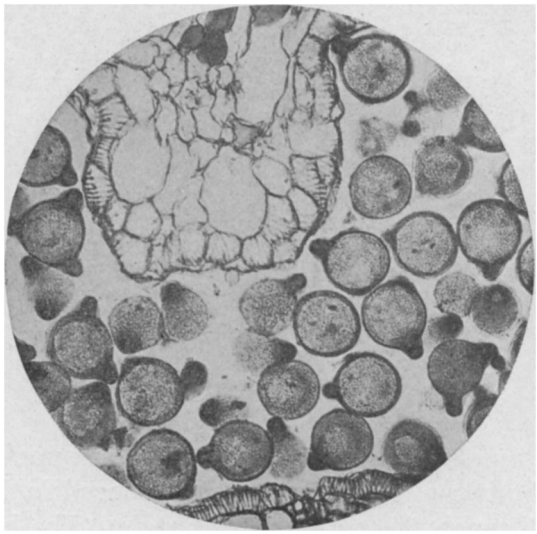

FIG. 3

that the pollen is perfect and invariable in any important respect. *E. angustifolium* is a species which apparently is not known to hybridize with other species and indeed it is not easy to see how it could cross with those having their pollen grains in tetrads. The perfection of the pollen in view of this condition appears particularly significant. The failure of *E. angustifolium* to hybridize in nature with other species of the genus is doubtless due to the fact that it is morphologically very distinct from these and would in all probability produce, if artificially crossed, only sterile hybrids.

We may now turn by way of comparison to a species of *Epilobium* of the ordinary type. Fig. 4 illustrates

photographically the floral organization of *Epilobium hirsutum,* as seen in transverse section of the bud just about to open. The illustration shows the floral envelopes

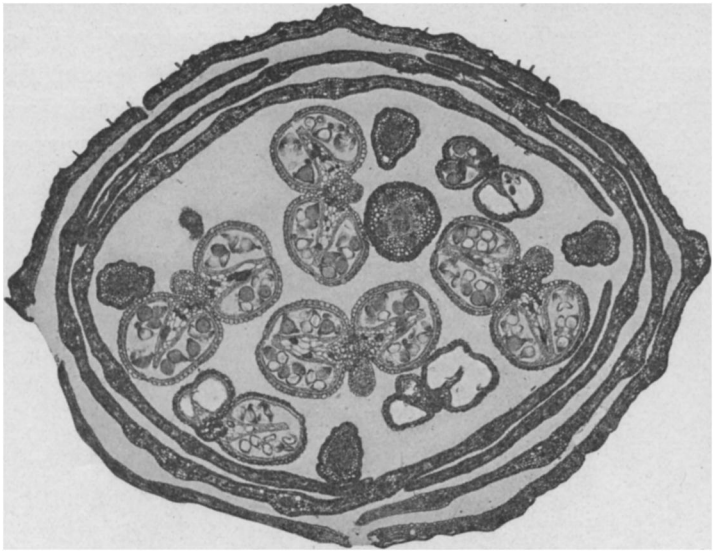

FIG. 4

and the stamens, together with the pistillary portion of the flower, the latter being somewhat displaced in the figure and cut through the region of the style. The long hairs characteristic of the calyx of this species have been trimmed off, for the purpose of facilitating photo-mechanical reproduction. As in the two illustrations above, the anther sacks are the most significant feature. Even with the low magnification employed for the purpose of illustrating the whole flower, the pollen grains in the loculaments of the anthers are easily discernible and present a striking contrast to those of *E. angustifolium,* in the respect that they are in groups of tetrads. Some of the groups are partially or wholly made up of individual grains without protoplasmic contents, which are smaller in size than the normal grains. Fig. 5 shows one of the anthers much more highly magnified. The anther walls, cavities and the pollen grains are now clearly distinguish-

able. Some of the grains are full size and present dark contents. Others are considerably smaller and are devoid of protoplasm. The latter are abortive or sterile grains. We have in fact before us a hybrid derivative of *E. hirsutum,* commonly found near ballast in New England and not unfrequently cultivated in gardens. Other species of *Epilobium* in the stricter sense of the generic appellation, show similarly abortive pollen development and the conclusion reached by old world systematists on the external

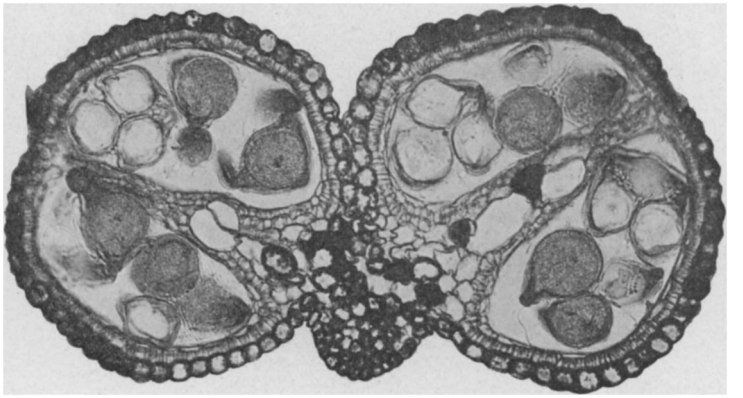

FIG. 5

characters, that hybridization is common among the species of *Epilobium* proper, is entirely confirmed by the study of the pollen. It need hardly be emphasized in this connection, that imperfect pollen development has been recognized for nearly a century by scientific plant breeders, as a criterion of hybrids.

The genus *Œnothera* may now be profitably considered. Fig. 6 presents a magnified view of a transverse section of a mature flower bud of one of the commonest of eastern species of *Œnothera,* namely *Œnothera biennis.* The floral envelopes are more voluminous than in the two genera illustrated above. Within are the stamens and in the center of the figure the style appears as a large rounded structure. Even with the low magnification employed, it is easy to discern that the contents of the anther sacks present a very different appearance from those of

Epilobium angustifolium. Many of the grains of pollen are light colored and devoid of the protoplasm which gives a dark appearance to the sound grains. Fig. 7 illus-

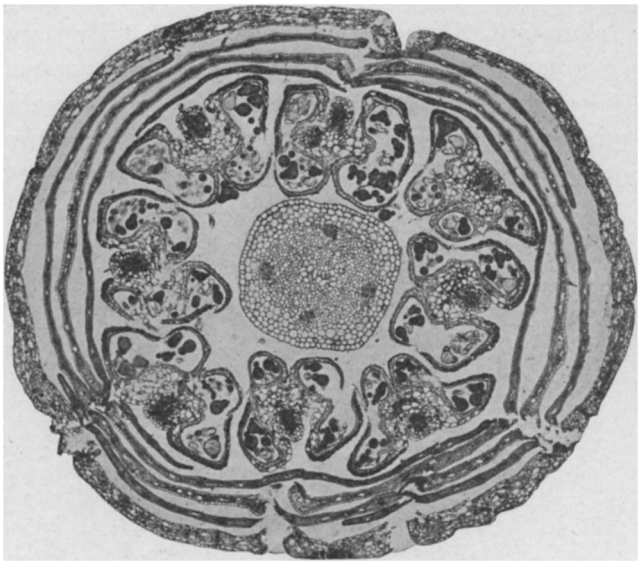

FIG. 6

trates a single stamen under a high degree of magnification. The characteristic layers of the wall of the anther sack, described comparatively and in detail in the classic memoir of Chatin, can readily be distinguished. Within lie the pollen grains. Clearly only a few of these are fully developed and possess normal protoplasmic contents. The greater number are shrivelled and empty. Judged from the generally accepted canon of the abnormalities of hybrids, *O. biennis* is of hybrid origin. This view of its nature is in harmony with its wide degree of inconstancy throughout its very extended range. This feature is doubtless responsible for the fact that the genus *Œnothera* is at the present time undergoing considerable elaboration, on the part of systematists. I have satisfied myself that the pollen peculiarities of *O. biennis* are uniformly present in specimens collected hundreds of miles apart, from the Province of Ontario, the shores of the Gulf of St.

Lawrence and the New England States. I have further examined a large number of species of *Œnothera* from various parts of the continent and in every instance have found a greater or smaller amount of abortive pollen as a characteristic feature of the anther contents. De Vries in his "Mutationstheorie" describes the abortive condition of about one third of the grains in *O. lamarckiana*. This feature has been seized upon with insight by Bateson, as indicating the hybrid origin of *O. lamarckiana*. It is extremely curious that its significance should have

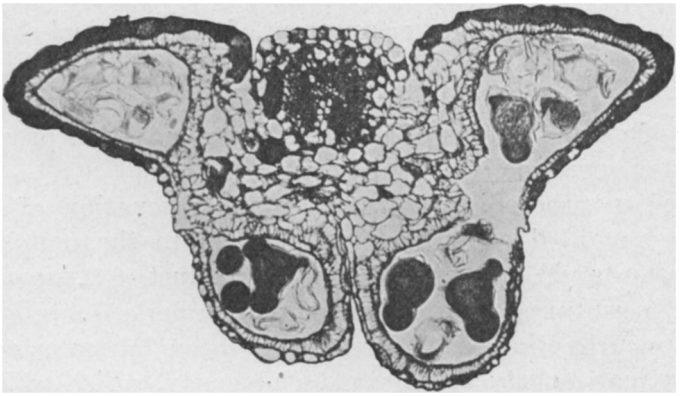

FIG. 7

escaped De Vries and his numerous disciples on this continent. Not only is *O. lamarckiana* itself characterized by a large proportion of abortive pollen but its so-called mutants are similarly characterized. In the feebler "elementary species" the pollen is often almost entirely abortive (*O. nanella*) and this is also generally the situation in *O. lata*. It should be further noted in this connection that if *O. lamarckiana* is of hybrid origin, the same statement must hold of the other species of *Œnothera*, since like this much-disputed one, they are similarly characterized, so far as they have been studied, by two correlated features, namely more or less abortive pollen and the peculiarity of throwing so-called mutants or "elementary species" in cultures. As a consequence of this condition,

it becomes more or less a superfluity to study any particular species of *Œnothera* from the genetical and morphological standpoint, since it is the genus as a whole which manifests the peculiar features, which have brought it so much into the foreground of biological controversy during the past decade. This is on the whole a satisfactory situation as it enables us to cut the perplexing gordian knot involving the controverted origin of *O. lamarckiana*. The mutation hypothesis of De Vries accordingly turns not upon the finding of new herbarium specimens which may throw light upon the origin of a particular species but upon the much larger question of the genetical status of the genus *Œnothera* as a whole. This question can be settled only by consideration of the Onagraceæ as a whole and of other families of the Angiosperms, which present similar reproductive peculiarities.

Before proceeding however to the discussion of the facts recorded above in their relation to the mutation hypothesis of De Vries, based on the conduct of *O. lamarckiana* in cultures, it will be necessary to make some brief reference to other studies carried on in the laboratories of plant morphology of Harvard University, which will be published elsewhere, either at the present time or at a later period. Obviously of great importance in the present connection is a comparison of the conditions of sporogeny found among the lower plants, the Bryophyta, the Pteridophyta and Gymnosperms, which are not characterized by enormous multiplication of species, with the sporogenic features of the Angiosperms in which the multiplication of species has run riot. Further comparison of liverworts, belonging to the Marchantiales, Anthosperms, manifesting similar sporogenic and specific peculiarities, is both pertinent and necessary, in the present connection.

It will be convenient to deal first summarily with the sporogenic conditions found in the lower forms of the Embryophyta from the Bryophyta to the Gymnosperms. In the present connection a considerable number of spe-

cies of liverworts, belonging to the Marchantiales, Anthocerotales and Jungermanniales, both acrogynous and anacrogynous have been examined with the general result that the only sterile cells present in the capsule cavities were the elaters. Infertile spores and hybridism both were conspicuous by their absence in the forms studied. The same statement *mutatis mutandis* holds for the true mosses. Some indication of spore abortion was detected in the extremely variable genus *Sphagnum*. It would seem that natural hybrids exist to some extent in this genus. Among the Pteridophyta both the Lycopsida and Pteropsida were studied. None of the numerous Lycopsid forms investigated showed signs of spore abortion or hybridism. Among the Pteropsida, the only well-known hybrids are found among what is probably the highest family, the Polypodiaceæ. There is a considerable literature upon hybrid ferns, in which references to spore abortion as an accompanying feature are common. No evidence of hybridism in the form of abortive spores was found in examples of the Marattiaceæ, Ophioglossaceæ, Osmundaceæ, Gleicheniaceæ, etc., were found, although a large amount of material was examined. Among the Gymnosperms, the Cycadales, Ginkgoales, Coniferales and Gnetales were examined. The Coniferales yielded only a single species of *Abies,* which showed evidence by the presence of abortive pollen grains of hybrid origin. The genus *Pinus* is very old and its species accordingly very distinct. Not the slightest evidence of hybridization was found here or in other numerous and widely distributed species of conifers, other than *Abies* mentioned above. This does not of course preclude the discovery of such conditions later. The writer has had the opportunity of examining the spores of a number of fossil forms from the Paleozoic and Mesozoic, still contained within the sporangia, and in no case were abortive spores recognized. The general conclusion can be drawn from the forms just considered that hybridism is rare among them and that

where it occurs it is accompanied by the phenomenon of spore abortion.

If we turn to the Angiosperms with their nearly one hundred and fifty thousand recognized species, we find that hybridism is very commonly recognized. It would take us much too far to discuss the situation here at any length. The consideration of a single important family must suffice. The one chosen, as being of particular significance in the present connection, is the Rosaceæ. We have had a recognition for many years past on the part of systematic botanists in this country and in Europe that hybridism is extremely common as a natural condition in certain genera of the Rosaceæ. The inference in such cases is generally based on the blended character of the hybrids themselves, which show to a large extent a combination of the characters of their parent species. Professor Brainerd has recently made some very interesting investigations in this direction in the case of American representatives of the Rosaceæ. The recognized hybrid forms in the Rosaceæ are usually characterized by a considerable degree of pollen sterility, unless the parents happen to be species not very remote in relationship. In addition to the recognized hybrids of the rosaceous species, the work carried on in the Harvard laboratories has revealed a large number of hidden hybrids or crypthybrids, which are quite constant in their characters and are recognized by systematists as good species, but differ from normal species in the fact that their reproductive cells are to a greater or less degree abortive. Species of this kind are extremely common among those rosaceous genera, which have become of economic importance, such as *Rubus, Rosa, Pyrus, Malus, Sorbus, Cratægus,* etc. Taking *Rosa* as an illustration, in addition to numerous recognized hybrids, there are many types recognized as good species, *e. g., Rosa blanda,* in which the pollen is normally largely abortive, in still other species, frequently those which are isolated geographically, the pollen is quite sound, *e. g., Rosa rugosa* of Japan. The latter type of species must be

regarded as a species in the strict sense, while those of the type of *Rosa blanda,* in which abortive pollen similar to that characteristic of forms clearly recognized as hybrids, is present, are hidden hybrids. It follows that in *Rosa* (or practically any of the other rosaceous genera cited above), there are three types of individuals, namely good species, hidden hybrids and open hybrids. The middle condition is extremely common among the Angiosperms and is of the greatest importance in connection with clear views in regard to the origin of species. Obviously constant or relatively constant hybrids can not rank with pure species, such as are characteristic for example of the Gymnosperms, in discussions in regard to the origin of species by mutation or otherwise. The conduct of such forms is conditioned to a greater or less extent by their mixed blood. We may appropriately designate obvious hybrids as phenhybrids and those hybrids which are recognizable as such by their internal morphological characters as crypthybrids. Crypthybrids will probably when studied more extensively in cultures by the geneticist, give evidence of their hybrid origin in cultures. There can be no doubt that many of the recognized species of the Angiosperms are in reality crypthybrids. The enormous multiplication of species in this great group of plants is in all probability largely related to hybrid crossing. It is of the utmost importance however to keep clearly in mind that such hybrid species or crypthybrids are not at all in the position of true species from the evolutionary standpoint and that conclusions derived from their study can not be applied without large reserves, to the question of the origin of species in the strict sense. The species of *Pinus,* so far as we have any evidence, since the main types are known to have existed well back into the Mesozoic, in all probability illustrate the origin of species somewhat along the lines of the Darwinian hypothesis. On the other hand the species of *Rosa* present obviously an entirely different problem in evolution and the necessity of making distinctions if we are to reach any definite bio-

logical goal is very clear. A great deal of the pessimism which at the present time is sending too many biologists after strange gods in other scientific shrines is doubtless to be traced to the failure to make this distinction. It may not be possible to make the distinction in all cases even among the higher plants; but it certainly will be necessary to realize its significance. Probably plants will in regard to this possibility enjoy in this respect, as in so many others, an advantage over animals in the studies of the experimental evolutionist.

We may now consider with advantage the status of the species of the genus *Œnothera*. The pollen sterility which characterized them all to a greater or less degree is indisputable evidence of their probable hybrid origin. The general situation in regard to the criteria of hybridism in plants has been recognized for nearly a hundred years. It has been made clear by Bateson in regard to *Œnothera lamarckiana*. The observations chronicled here appear to make it obvious that all the species of *Œnothera* are in the same boat genetically, that is that they are all of hybrid origin. They likewise probably will all be found to "mutate" just as *O. lamarckiana, O. biennis,* etc., are already known to do. It may appear later that there are certain species which have escaped, through geographical isolation or other causes, the mingling of blood, which is certainly characteristic of the *Œnotheras* of the Eastern United States. So far as we know them at present, the species of *Œnothera* are obviously in the same position as such species as *Rosa blanda,* that is they are crypthybrids. Doubtless the peculiarities of *O. lamarckiana, O. biennis,* etc., can be more clearly explained in the present condition of our knowledge as the result of hybrid origin than in any other way. It follows that the doctrine of mutation so far as it depends for its support upon the *Œnotheras* is in a discredited condition, as an explanation, in any proper sense of the term, of the origin of species.

Conclusions

1. The Onagraceæ are largely characterized by hybrid contamination in nature.

2. This statement holds with particular force for *Œnothera lamarckiana* and other species of the genus *Œnothera,* which have served as the most important basis of the mutation hypothesis of De Vries.

3. Constant hybrids or crypthybrids are of very common occurrence among the Angiosperms and have been illustrated in the present article by reference to the genetical conditions occurring in certain Rosaceæ.

4. The species of *Œnothera* are to a large extent, if not wholly, crypthybrids.

5. The objection raised by Bateson to the genetical purity of *Œnothera lamarckiana* is confirmed and is extended to the Onagraceæ in a general way, as well as to other species of *Œnothera.*

6. Hybridism is the best explanation yet put forward of the peculiar conduct of *Œnothera lamarckiana,* as well as other species of the genus in cultures.

7. The mutation hypothesis of De Vries, so far as it is supported by the case of *Œnothera lamarckiana,* is invalidated.